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ONTOGENETICAL AND CYTOCHEMICAL STUDIES OF ALKALOIDAL VESICLES IN LATICIFERS OF PAPAVER SOMNIFERUM L. 2/

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1/ This ST/SOA/SER.J/.. series of documents was previously entitled "Scientific research on Papaver bracteatum".

2/ This study represents a portion of a Ph.D. thesis in the Department of Plant Sciences, Indiana University, Bloomington, Indiana, 47401, U.S.A.
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General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

INTRODUCTION

Laticifers in Papaver somniferum L. can be distinguished from other cell types at the ultrastructural level by the large irregularly shaped vesicles that fill the mature cell. Origin of these vesicles by the distention of endoplasmic reticulum was first suggested by Sárkány et al. (1964) and later by Thureson-Klein (1970), although vesicle ontogeny was not studied in detail. The occurrence of alkaloids in latex from poppy has been known from the classical literature and recently Fairbairn et al.(1974) reported that the alkaloids were associated with the vesicles within the vacuolar plasm of this cell. Dickenson and Fairbairn (1975) investigated the ultrastructure of these "alkaloidal vesicles" in both ultracentrifuged latex and intact plants.

In the present investigation the ontogeny of alkaloidal vesicles in laticifers of P. somniferum is described and cytochemical evidence presented for their origin from endoplasmic reticulum. Information derived from our current research program will contribute to a more complete understanding of the laticifer and its latex, and will contribute to our understanding of the mechanism of alkaloid formation whereby we may be able to increase the yield of alkaloids in this plant.

MATERIALS AND METHODS

Plants of Papaver somniferum L. cv. "Neuga" were grown from seed in the greenhouse. Seeds were also germinated on cheese cloth covered paper cylinders in glass jars to obtain straight radicles for study. Various portions of plant axes were fixed 8 - 12 hr at room temperature in one of the following fixatives: 1) 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2; 2) 1% glutaraldehyde, 3% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2 (Karnovsky, 1965); or 3) 4% glutaraldehyde, 0.7% picric acid in 0.1 M sodium cacodylate buffer, pH 7.0 (Turner, 1970). After several rinses with 0.05 M sodium cacodylate, pH 7.0, tissues were post-fixed in 1% osmium tetroxide in 0.05 M sodium cacodylate, pH 7.0, for 2 hr, rinsed with glass-distilled water and prestained in 1% aqueous uranyl acetate at 4 C for 6 - 12 hr. Tissues were

then rinsed with water, dehydrated through an ethanol-acetone series and embedded in Spurr (1969) resin. Some tissues were fixed in 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.2 for 2 hr and dehydrated and embedded as described above.

Procedures described above were modified for cytochemical electron microscopic investigations as follows:

1) Zinc iodide-osmium tetroxide (ZIO) impregnation. Tissues were fixed in the dark for 24 hr in a zinc iodide-osmium tetroxide solution (Niebauer et al. 1969). Tissues were rinsed with water, prestained with uranyl acetate, dehydrated and embedded as described above. A modification of this procedure consisted of treating tissues with Turner (1970) fixative for 8 - 16 hr before ZIO impregnation.

2) Osmium tetroxide impregnation. Tissues were fixed at room temperature in 1% osmium tetroxide in 0.05 M phosphate buffer, pH 7.2, for 1½ hr. After initial fixation, tissues were impregnated for 2 - 6 days with osmium tetroxide by placing them in foil covered vials containing unbuffered 2% osmium tetroxide at 40 C (Friend, 1969). Osmium tetroxide solutions were changed every 24 hr. Tissues were rinsed with water and prestained with unranyl acetate for 1½ hr at room temperature, dehydrated and embedded as described above. Thin sections were cut on a Porter-Blum MT-2 ultramicrotome, collected on 300 mesh copper grids, stained with lead citrate and unranyl acetate and examined with a Hitachi HU-11C electron microscope at 50 KV.

RESULTS

In early stages of differentiation, rough endoplasmic reticulum was abundant throughout the densely packed cytoplasm of laticifer initials. During laticifer differentiation this endoplasmic reticulum accumulated along the periphery of the plasma membrane in long folded sheets oriented parallel to the longitudinal wall (Fig. 1). Segments of this endoplasmic reticulum also occurred within a lobe of the nucleus (Fig. 4) and in smaller sheets surrounded by developing vesicles (Fig. 5).

Endoplasmic reticulum of cells in early stages of vesiculation were represented by rough endoplasmic reticulum alternating with smooth areas that lacked ribosomes (Fig. 2). Stacked endoplasmic reticulum was surrounded by high concentrations of

polysomes that appeared to be derived from the surface of rough endoplasmic reticulum as it was transformed to the smooth form (Fig. 1, 2).

Vesicles, which comprised most of the mature laticifer protoplast, were bounded by unit membrane (Fig. 5) and appeared to be derived from endoplasmic reticulum. Vesicles were derived from dilation of both smooth (Fig. 6) and rough (Fig. 7) endoplasmic reticulum, the latter losing ribosomes from its surface during dilation. Derivation of vesicles from dilation of endoplasmic reticulum is illustrated in Fig. 23.

Impregnation with the zinc iodide-osmium tetroxide (ZIO) reagent resulted in differential staining of laticifer vesicles as well as the vacuolar membrane of neighbouring cells (Fig. 8, 9). This staining resulted in selective deposition of granular electron-dense materials along the inner surface of these membranes. When ZIO impregnation was preceded by fixation with glutaraldehyde in picric acid, the ultrastructure of all cells was well preserved (Fig. 10). With this treatment, laticifer vesicles were selectively stained along their inner membrane (Fig. 11); adjacent cells showed occasional staining only within the perinuclear space and mitochondrial matrices (Fig. 12).

Laticifer vesicles (Fig. 13, 14) and endoplasmic reticulum (Fig. 14, 15) were selectively stained by osmium tetroxide impregnation. The forming face of dictyosomes and portions of endoplasmic reticulum in adjacent cells were also differentially stained (Fig. 16). As with the ZIO technique, only the inner membrane surface of vesicles was stained (Fig. 17).

As vesicles began to proliferate in the laticifer cytoplasm, black electron-dense regions or "caps" developed along their periphery (Fig. 19) by the condensation of small particles along the inner surface of the vesicle membrane (Fig. 1). These particles, which formed within the developing vesicles, appeared as a mixture of small electron-dense and electron transparent granular deposits at high magnification (Fig. 18). The relationship between these different particle types is not known at present.

Condensation of particles into caps occurred as vesicles enlarged (Fig. 1); however, the caps were formed before the laticifer was fully differentiated (Fig.19).

When tissue at the late vesiculation stage was fixed in buffered osmium tetroxide, the vesicle caps appeared enlarged and granular (Fig. 20, 21). Vesicle caps were not as conspicuous in mature laticifers as in earlier stages of development of this cell (Fig. 3, 22). The large size and close packing of vesicles in the mature laticifer protoplast made the caps difficult to recognize.

The origin and development of vesicle caps during the vesiculation process in the developing laticifer is illustrated in Fig. 23.

DISCUSSION

Results of our cytochemical investigation support the interpretation that all vesicles of the mature laticifer protoplast were derived from endoplasmic reticulum. These studies confirm the observations of Thureson-Klein (1970) who reported that vesiculation resulted from localized dilation of endoplasmic reticulum.

All the vesicles of mature laticifers in opium poppy are stained by ZIO impregnation as is the vacuolar membrane of neighbouring cells. The stain appears as electron-dense deposits along the inner surface of these membranes and is similar to the description reported by Marty (1973a, b) for the vacuolar membrane and provacuoles of Euphorbia characias cells.

Results of ZIO impregnation indicate that laticifer vesicles in poppy may be analogous to the central vacuole of other cells as suggested by Thureson-Klein (1970), since both membranes show a similar staining pattern with this procedure. However, this procedure does not conclusively demonstrate that these membranes originate directly from dilation of endoplasmic reticulum in view of the similar staining of dictyosome-derived vesicles reported by Dauwalder and Whaley (1973) and Marty (1973a, b).

In plants, fixation with glutaraldehyde followed by ZIO impregnation has been reported to stain the endoplasmic reticulum, proximal face of dictyosomes,

nuclear envelope, mitochondrial matrices and occasional plastid matrices (Dauwalder and Whaley, 1973; Susalla and Mahlberg, 1975). Application of this technique to opium poppy tissue resulted in the staining of all laticifer vesicles and occasional staining of the perinuclear space and mitochondrial matrices of adjacent cells.

Differential staining of laticifer vesicles and the nuclear envelopes of adjacent cells would seem to directly implicate the endoplasmic reticulum in vesicle formation, but such staining has also been reported for dictyosome vesicles in cortical parenchyma of Zea mays (Dauwalder and Whaley, 1973).

In other studies on plant cells, the ZIO technique has been found to stain the endoplasmic reticulum, nuclear envelope, and tonoplast of some impregnated cells (Dauwalder and Whaley, 1973; Marty, 1973a, b). The internal portions of lamellar membranes of chloroplasts are also differentially stained by ZIO (Dauwalder and Whaley, 1973; Susalla and Mahlberg, 1975) as well as the photosynthetic "organelles" of purple bacteria (Turner et al. 1973). Staining of dictyosomes appears to be more variable. In Zea mays root apices, Dauwalder and Whaley (1973) reported the proximal face of dictyosomes stained with ZIO in cells of the epidermis and rootcap, whereas in cortical parenchyma of Euphorbia characias both distal and proximal faces of dictyosomes stained, while several internal cisternae remained unstained (Marty, 1973b). Marty (1973a, b) concluded that dictyosomes play a role in the production of vacuoles in various cells in addition to their known secretory function.

The osmium tetroxide technique when applied to poppy laticifers stained both endoplasmic reticulum and vesicles. Adjacent cells showed staining of endoplasmic reticulum and the proximal face of dictyosomes.

In other plant tissue, impregnation with osmium tetroxide differentially stained the nuclear envelope (Dauwalder and Whaley, 1973; Chardard, 1973; Noguchi, 1976), vacuoles (Chardard, 1973; Noguchi, 1976), tannin vacuoles (Poux, 1973), internal pro-plastid membranes (Dauwalder and Whaley, 1973; Noguchi, 1976; Poux, 1973), mitochondria (Noguchi, 1976) and the proximal face of dictyosomes (Micalef, 1975; Noguchi, 1976; Poux, 1973).

Results derived from the osmium tetroxide impregnation procedure coupled with staining patterns previously reported for this technique support the hypothesis that laticifer vesicles are derived from dilation of endoplasmic reticulum.

Dickenson and Fairbairn (1975) described electron-dense regions or caps associated with alkaloidal vesicles and proposed that capped vesicles represented the mature form of previously "granulated" non-capped vesicles. Reports from earlier investigations are contradictory on this point in that caps have been described as occurring in young laticifers (Schulz, Schnepf and Mothes, 1967) and also fully differentiated cells (Thureson-Klein, 1970).

The developmental sequence described in the present study indicates that capped vesicles are most abundant and most conspicuous in intermediate states of laticifer differentiation. Capped vesicles appear to represent a transitional form between the small capless vesicles of immature laticifer elements and the large irregularly shaped vesicles characteristic of mature cells. The dense packing of enlarged capped vesicles at maturity obscures the caps and probably contributes to their granular appearance.

CONCLUSIONS

Development of alkaloidal vesicles in laticifers of opium poppy, Papaver somniferum L. was investigated at the ultrastructural level. Laticifer initials possessed abundant endoplasmic reticulum throughout their dense cytoplasm. During differentiation, the endoplasmic reticulum organized into long folded sheets that were parallel to the longitudinal walls along the periphery of the cell. Vesicles appeared to be derived from dilation of endoplasmic reticulum. This relationship was confirmed through cytochemical data obtained with zinc iodide-osmium tetroxide and osmium tetroxide impregnation. Alkaloidal vesicles had electron-dense regions or caps that occurred early in laticifer differentiation, but these caps became less conspicuous in mature cells. Caps appeared to be derived from small electron-dense and electron transparent particles which condensed along the inner surface of the vesicle membrane and subsequently accumulated at one or two positions along the membrane of the vesicle.

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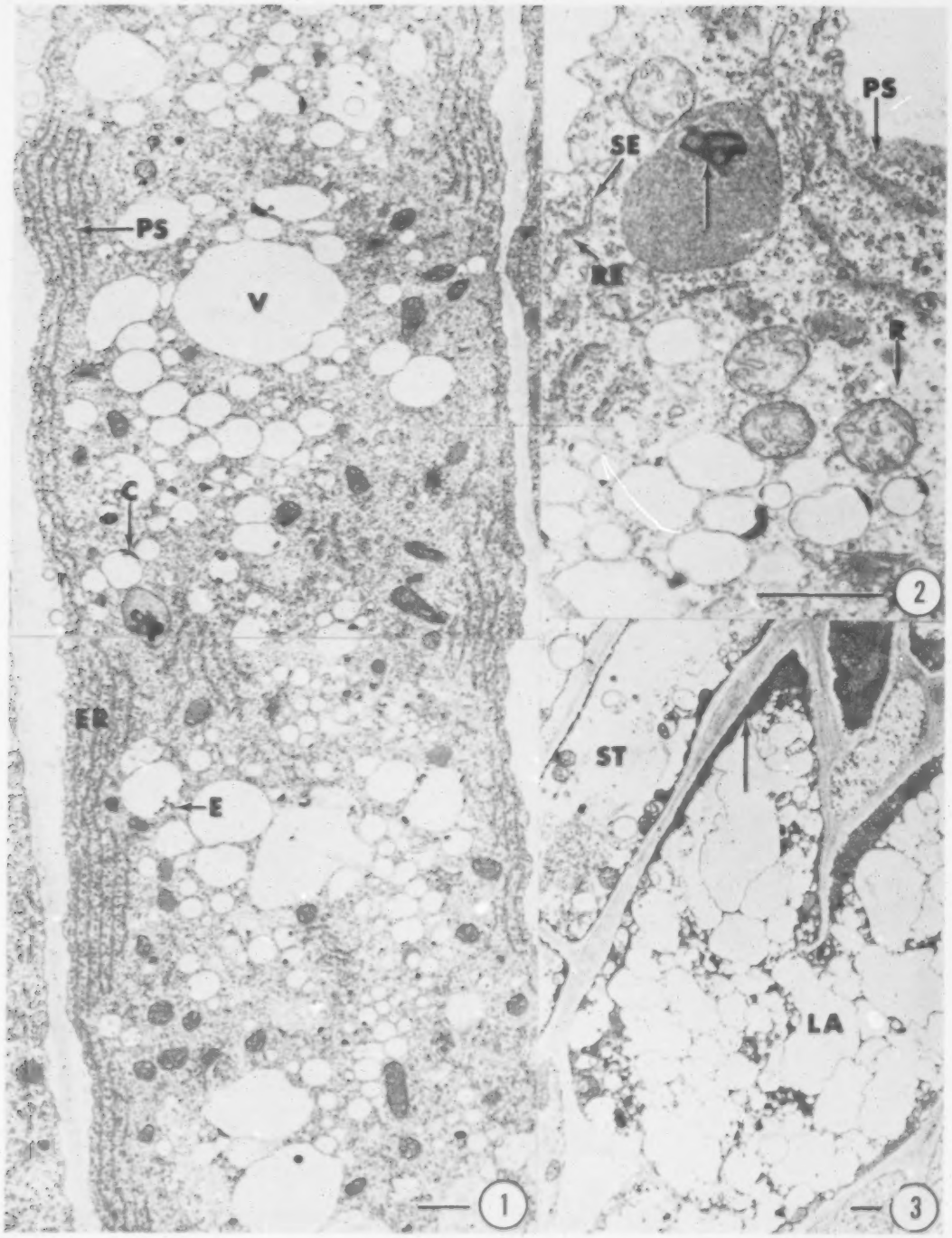
Figures 1 - 23

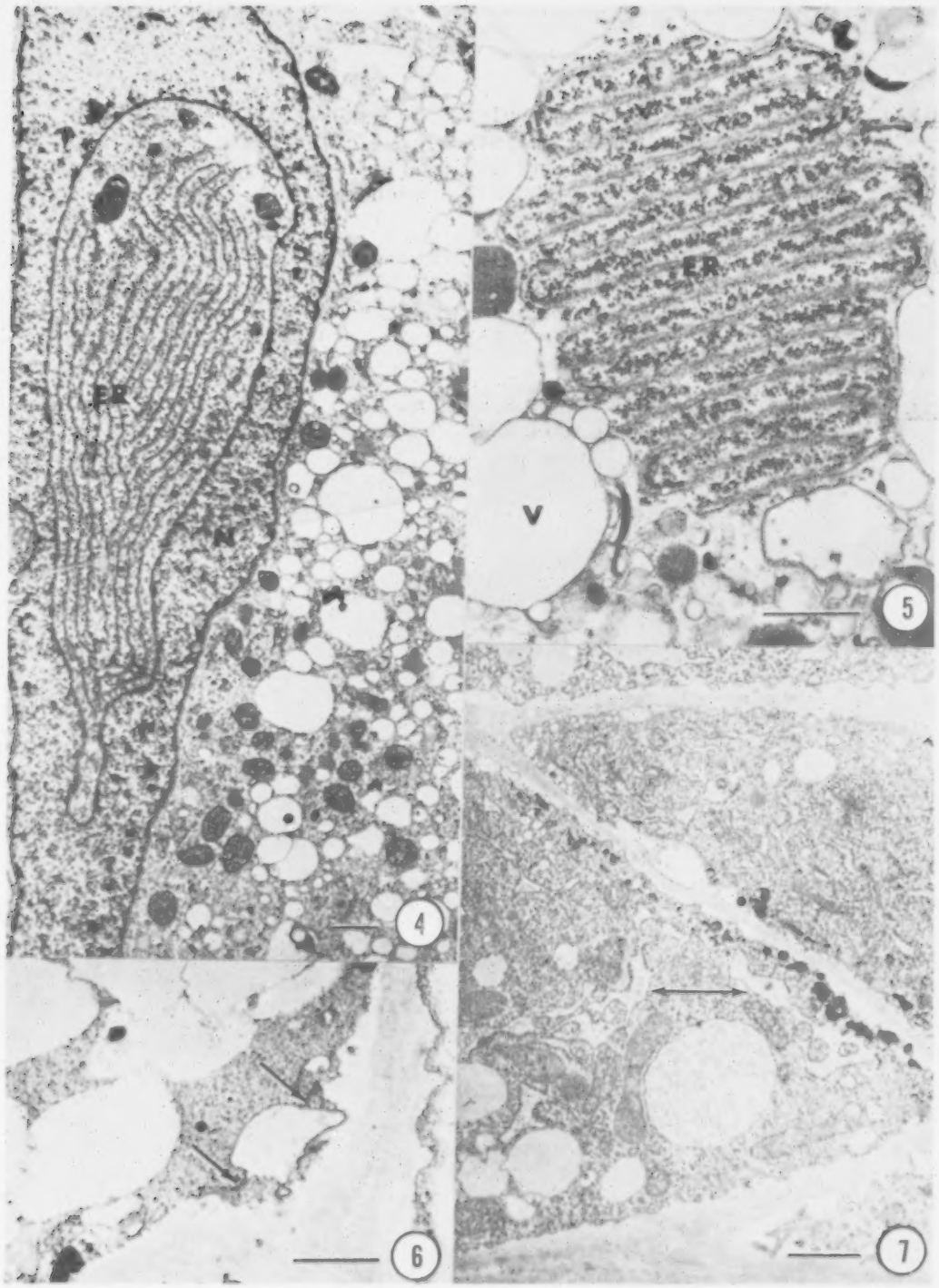


Figures 1-2: Laticifers in P. somniferum.
Fixation: Karnovsky/OsO_4. Magnification line represents 1 µm.

Figure 1 - Laticifers in early vesiculation stage of development from 50 hr radicle showing polysomes (PS) concentrated in regions of stacked endoplasmic reticulum (ER). C: cap; E: electron dense particles; P: plastid; V: vacuole.

Figure 2 - Laticifers in early vesiculation stage of development from 35 hr radicle showing polysomes (PS) and smooth endoplasmic reticulum (SE) alternating with rough endoplasmic reticulum (RE). Arrow: plastid core; R: ribosomes.

Figure 3 - Mature laticifer (LA) from leaf midvein adjacent to sieve-tube member (ST). Arrow: peripheral cytoplasm.



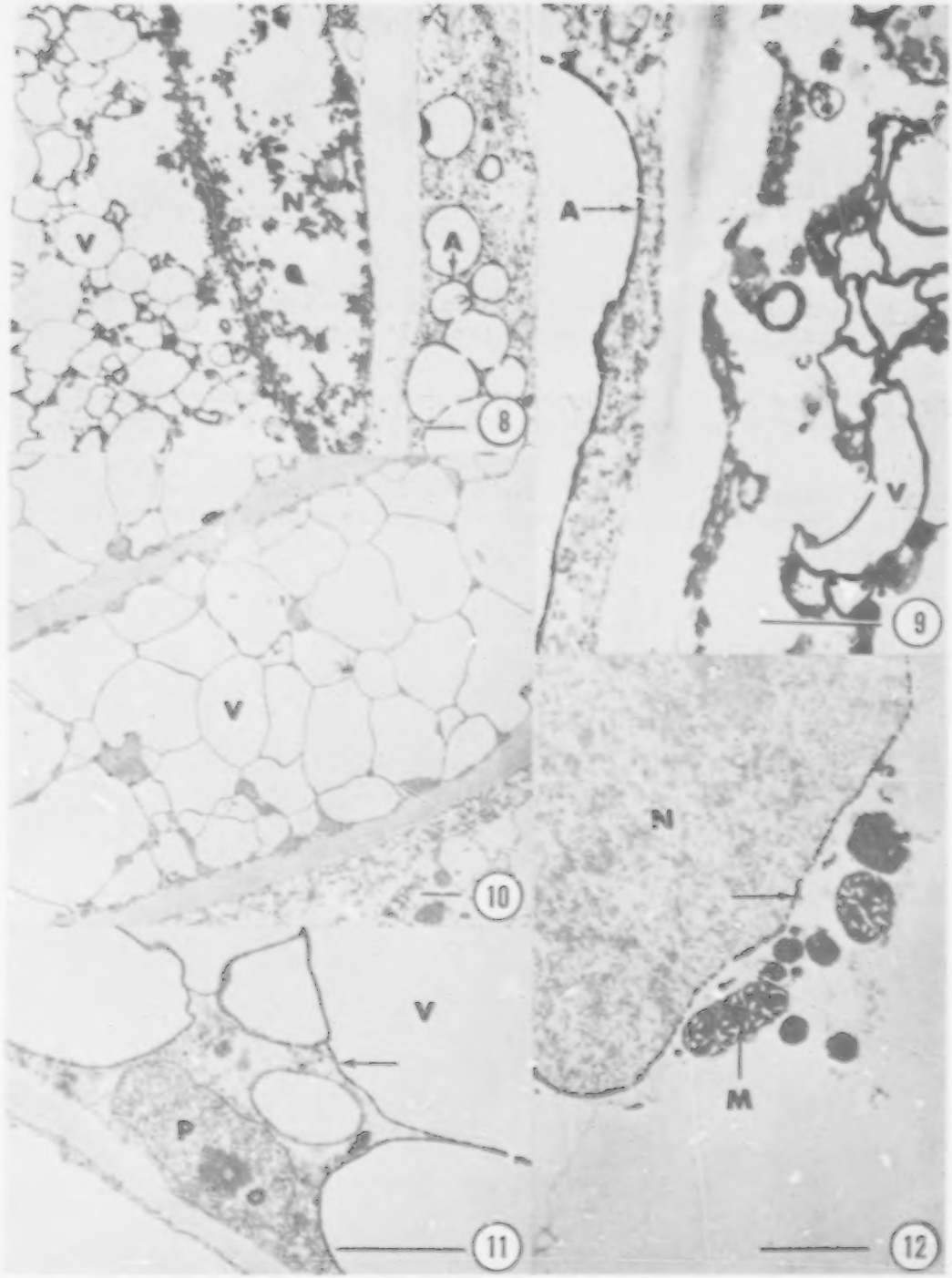
Figures 4-7: Laticifers in P. somniferum. Fixation: Fig. 4, 6, 7 Karnovsky/OsO_4; Fig. 5, 2% glutaraldehyde/OsO_4. Magnification line represents 1 µm in Fig. 4, 6, 7 and 0.5 µm in Fig. 5.

Figure 4 - Laticifer in early vesiculation stage of development from 50 hr radicle showing stacked endoplasmic reticulum (ER) within a lobe of the nucleus (N).

Figure 5 - Laticifer from shoot apex showing stacked endoplasmic reticulum (ER) surrounded by developing vesicles (V).

Figure 6 - Laticifer from the hypocotyl region showing vesicle formation by dilation of smooth endoplasmic reticulum (between arrows).

Figure 7 - Laticifer from 90 hr radicle showing vesicle formation by apparent dilation of rough endoplasmic reticulum (arrow).



Figures 8-12: Tissues from P. somniferum impregnated with zinc iodide-osmium tetroxide (ZIO). Magnification line represents 1 µm.

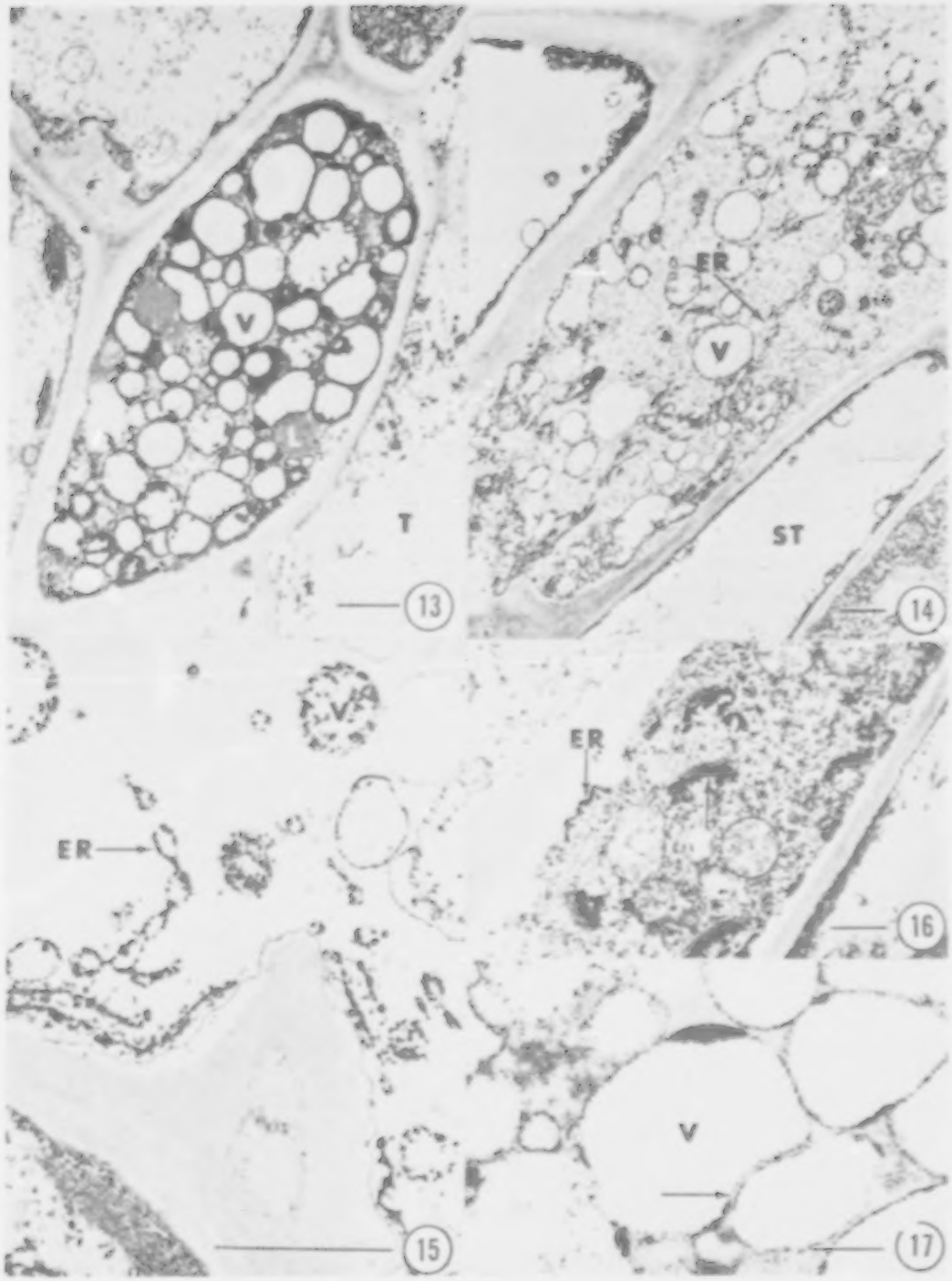
Figure 8 - Mature laticifer from leaf midvein with stained vesicles (V) and adjacent parenchyma with stained tonoplast (A) after ZIO impregnation. N: nucleus.

Figure 9 - Mature laticifer from shoot apex with stained vesicles (V) and adjacent parenchyma with stained tonoplast (A) after ZIO impregnation.

Figure 10- Laticifers of the petal with stained vesicles (V) after fixation in Turner's fixative followed by ZIO impregnation.

Figure 11- Laticifers of the petal showing differential staining of vesicular membranes (arrow) after fixation in Turner's fixative followed by ZIO impregnation. P: plastid; V: vesicle.

Figure 12- Non-laticiferous cell from 96 hr radicle showing differential staining of the perinuclear space (arrow) and mitochondrial matrices (M) after fixation in Turner's fixative followed by ZIO impregnation. N: nucleus.



Figures 13-17: Tissues from P. somniferum impregnated with osmium tetroxide. Magnification line represents 1 µm in Fig. 13-15, 2 µm in Fig. 16 and 0.5 µm in Fig. 17.

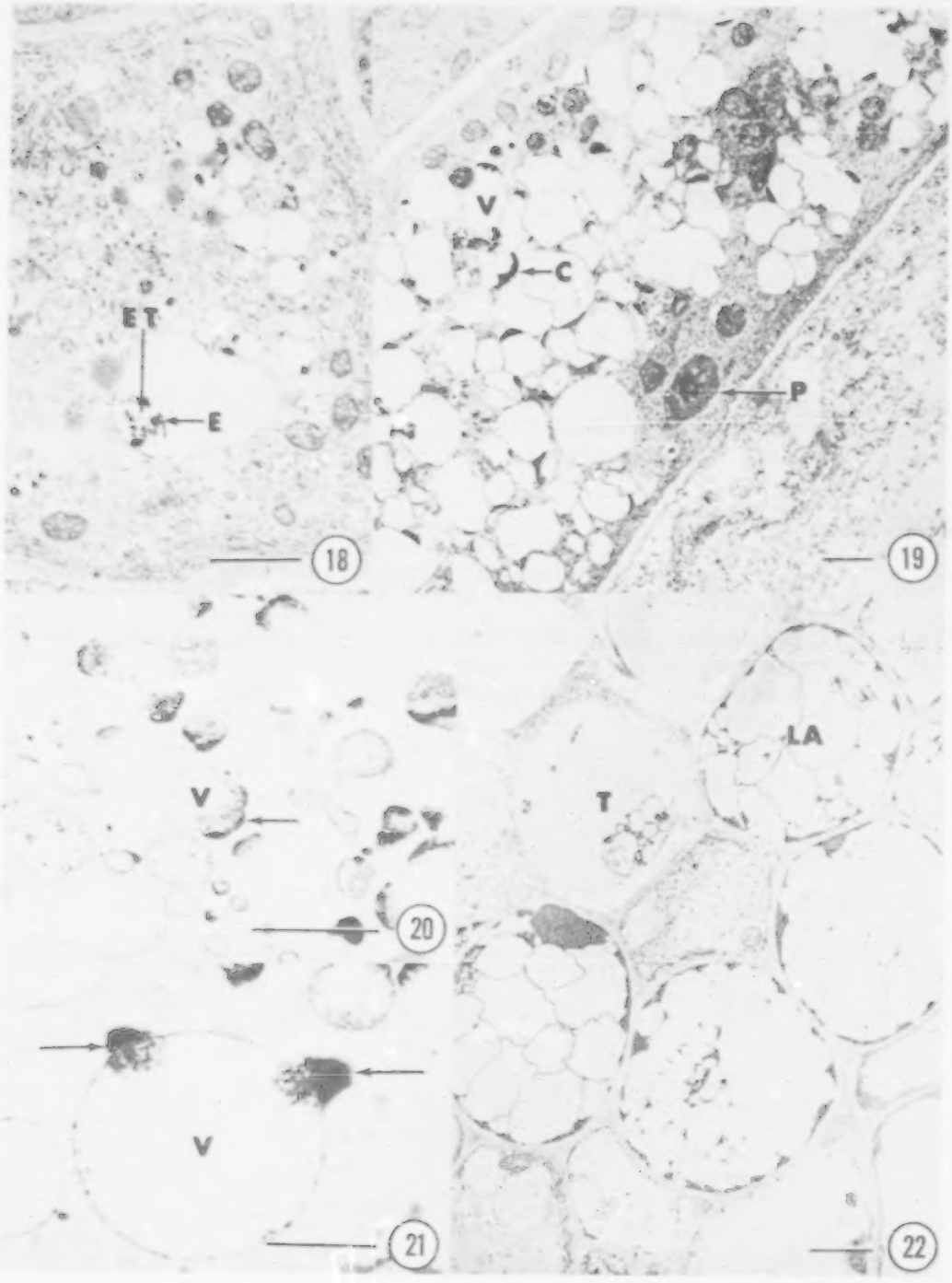
Figure 13 - Mature laticifer from first seedling leaves showing differential staining of vesicles (V) after osmium tetroxide impregnation. L: lipid droplet; T: tracheary element.

Figure 14 - Laticifer in early stage of vesiculation from first seedling leaves showing differential staining of vesicles (V) and dilating endoplasmic reticulum (ER) after osmium tetroxide impregnation. ST. sieve-tube member.

Figure 15 - Mature laticifer from leaf midvein showing differential staining of vesicle (V) and dilating endoplasmic reticulum (ER) after osmium tetroxide impregnation.

Figure 16 - Non-laticiferous cell from leaf midvein showing differential staining of the forming face of dictyosomes (arrow) and endoplasmic reticulum (ER) after osmium tetroxide impregnation.

Figure 17 - Laticifer vesicle (V) from shoot apex showing differential staining along the interior of its membrane (arrow) after osmium tetroxide impregnation.



Figures 18-22: Laticifers in P. somniferum. Fixation: Fig. 18, 19 Karnovsky/OsO_4; Fig. 20, 21 OsO_4; Fig. 22 Turner/OsO_4. Magnification line represents 1 µm in Fig. 18-20, 0.5 µm in Fig. 21 and 2 µm in Fig. 22.

Figure 18 - Laticifer in early stage of vesicle formation from 35 hr radicle showing the accumulation of electron-dense (E) and electron-transparent (ET) particles into the vesicle cap.

Figure 19 - Laticifer in late stage of vesicle formation from 100 hr radicle showing the abundant electron-dense caps (C) at the periphery of vesicles (V). P: plastid.

Figure 20 - Laticifer vesicles (V) with granulated cap (arrow) in seedling midvein fixed in osmium tetroxide.

Figure 21 - Laticifer vesicle (V) with two granulated caps (arrows) in seedling midvein fixed in osmium tetroxide.

Figure 22 - Cross section through a vascular bundle of the petal showing four latex vessels (LA) and an adjacent tracheary element (T).

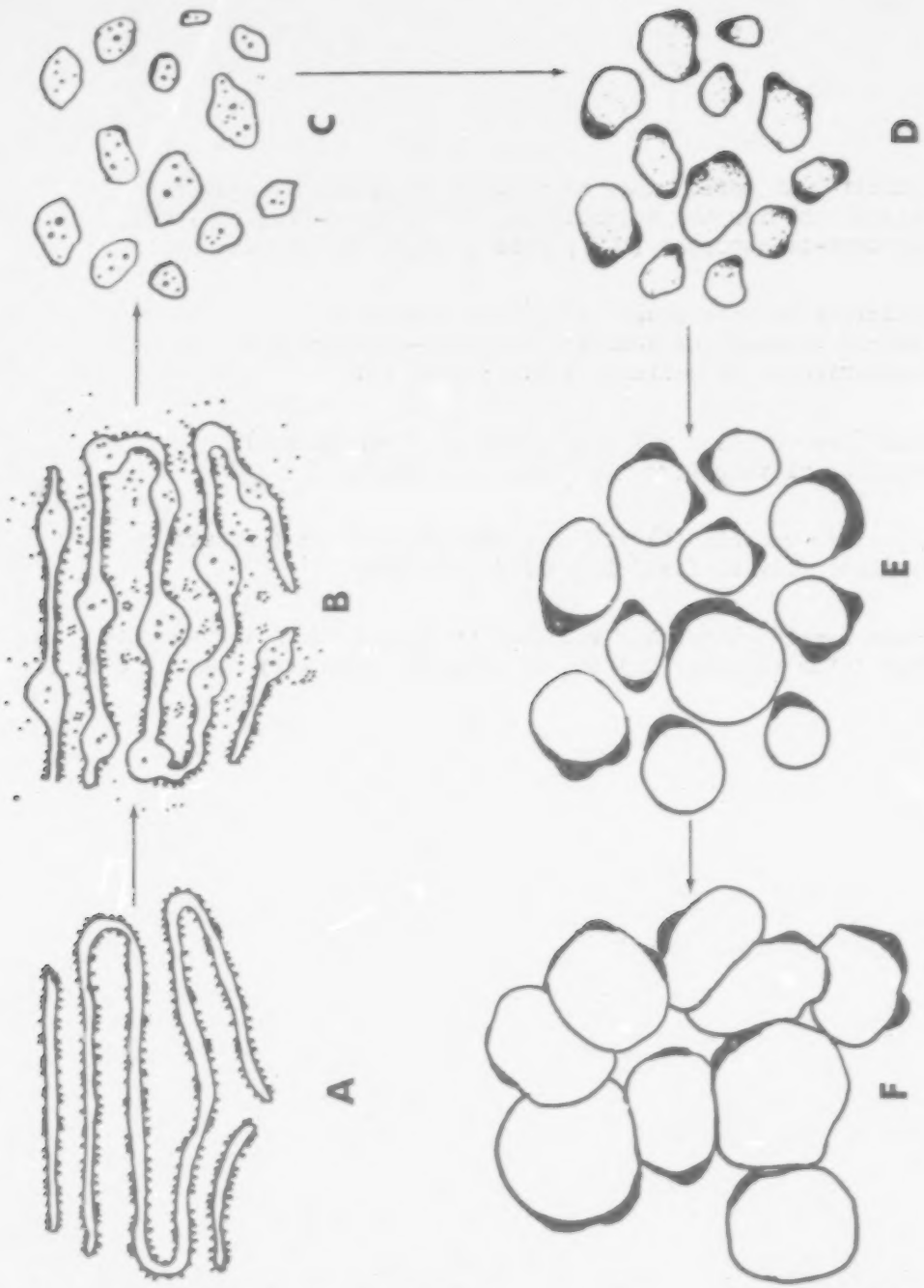


Fig. 23

Fig. 23 - Diagram of vesicle formation from dilation of endoplasmic reticulum. A. Stacked or unstacked endoplasmic reticulum. B. Dilation of endoplasmic reticulum with ribosomes. C. Small vesicles with electron-dense and electron-transparent particles. D. Concentration of particles along surface of enlarging vesicles. E. Capped vesicles, before complete vesicle expansion. F. Large irregularly shaped vesicles in mature cell. Caps obscured by the dense packing of vesicles.